Electrophoretic variation of Adenosine Deaminase (ADA) in pigs

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Introduction

Adenosine deaminase is involved in the deamination of adenine to inosine. In a variety of animal tissues and sera multiple molecular forms of adenosine deaminase have been demonstrated (Spencer et al. 1968).

Polymorphic variation in red cell ADA of humans was reported by Spencer et al. (1968). These variants were shown to be products of autosomally codominant alleles controlling the electrophoretic expression of red cell ADA. Rare variants at this locus have been reported in a variety of human populations, which were shown to represent heterozygous combinations for a common allele and a rare allele by family studies (Edwards et al. 1972).

Materials and methods

Samples of blood were obtained from the slaughter house in Wiesbaden. Serum was separated by centrifugation and red cell haemolysates were prepared by washing the red cells three times in saline and haemolysed by the addition of an equal volume of distilled water.

Starch gel electrophoresis was the method of choice, using the buffer system of Kirchberg and Wendt (1970). After electrophoresis lasting 16—18 hours at a voltage gradient of 120 volts, the ADA pattern was localised using the staining technique of Spencer et al. (1968).

Results and discussion

Upon localisation of ADA activity, three patterns were observed. The most common pattern was found to consist of a single band of enzyme activity moving anodally as shown in the fig. Though the other two patterns were much les frequent, the patterns were reproducible. One of these had two bands, one in the same position as in the pattern described and another slower band about 2 cms from the faster moving band. The third pattern also consisted of a single band in the same position as the slower moving band of the second electrophoretic pattern. We have provisionally called the faster moving pattern as $ADA_{pig}1-1$, and the other two as $ADA_{pig}2-1$ and $ADA_{pig}2-2$ respectively, since all patterns were found to be considerably faster in mobility compared with the ADA pattern of humans.

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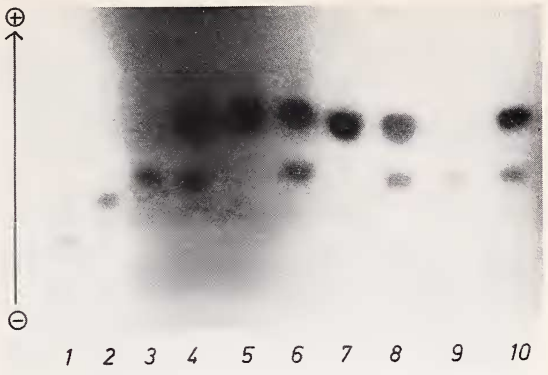

Starch gel electrophoretic pattern of human and pig red cell ADA. 1 and 2 are human red cell ADA, 2 = after incubation with GSSG. 3, 4, 5, 6 = pig phenotypes, 7, 8, 9, 10 = after incubation with GSSG. $ADA_{pig}1$–1 = 5 and 7, $ADA_{pig}2$–1 = 4 and 6 and $ADA_{pig}2$–2 = 3 and 9

Omission of Adenine or Arsenate from the reaction mixture was found to have a profound effect in that no enzyme bands were seen. However, the omission of either Nucleoside phosphorylase or Xanthine oxidase was hardly found to affect the patterns though the diffusion of the bands was noted. Spencer et al. (1968) noted hazy bands of enzyme activity after the omission of Nucleoside phosphorylase but not Xanthine oxidase, suggesting that Nucleoside phosphorylase could be found in the cells, wich is possible in the case of the pig also. It is likely that Xanthine oxidase also could be found in the cells thereby explaining the observed phenomena, and need not necessarily imply an error in the identity of the enzyme.

Oxidised glutathione (GSSG) has been found to affect the mobility of the human red cell ADA thereby indicating the possible of a free sulfhydryl group on the surface of the molecule (Hopkinson and Harris 1969). Incubation of the pig red cells with about 2 mgms. of GSSG for one hour at 37° C did not have any effect on the electrophoretic mobility or on the relative concentration of the enzyme bands in all the three patterns (Fig. 1). The control human sample on the other hand, was found to behave as has been observed by Hopkinson and Harris (1969).

The observed variation could be genetic and the phenotypes do not contradict the existence of two codominant alleles producing the observed patterns in the proportions mentioned in the table. If the observed variation is genetic it gives an ADA_{pig}^{1} gene frequency of 0.948. However, pedigree studies are needed to confirm the present hypothesis.

Distribution of electrophoretic variants of Adenosine Deaminase (ADA) in the pig

No.	1—1	2—1	2—2
87	81	3	3
%	93.10	3.45	3.45

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Summary

Red cells from 87 pigs were tested for electrophoretic variants of Adenosine deaminase. Three phenotypes were found to occur with the following frequencies: $ADA_{pig}1–1 = 93.10\,\%$, $ADA_{pig}2–1 = 3.45\,\%$, and $ADA_{pig}2–2 = 3.45\,\%$. Incubation with oxidised glutathione was not found to affect the electrophoretic mobility.

Zusammenfassung

Elektrophoretische Variantew der Adenosindeaminase bei Schweinen

An 87 Blutproben von Schweinen wurden Untersuchungen über elektrophoretische Varianten der Adenosindeaminasen (ADA) durchgeführt. Es wurden drei Phänotypen gefunden mit den Häufigkeiten: $ADA_{pig}1–1 = 93.10\,\%$, $ADA_{pig}2–1 = 3.45\,\%$ und $ADA_{pig}2–2 = 3.45\,\%$. Inkubation von oxidiertem Glutathion scheint keinen Einfluß auf die elektrophoretische Mobilität der Banden zu haben.

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